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Activation of potassium channels: Relationship to the heat shock response

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ABSTRACT **We examined the possibility that whole cell currents are involved and possibly trigger the response of mammalian cells to heat shock. Heat-sensitive cells from a radiation-induced fibrosarcoma (RIF-1) and heat-resistant variants (TR-4, TR-5) were heated at 45°C for 3–30 min. We observed induction of voltage-dependent currents after heating in the heat-resistant cells. These currents decayed to nonmeasurable levels over a period of 6 h. In RIF-1 cells, however, voltage-dependent currents were detectable during heating only; these currents then decayed rapidly. Tetraethylammonium (TEA) cations blocked the currents; changing the concentration of extracellular K^+ modified the current–voltage (*I–V*) relationship. These currents, therefore, resulted from the activation of voltage-dependent K^+ channels. Addition of TEA during heating sensitized TR-4 cells to heat but had no effect on the heat response of the RIF-1 cells. Continuous exposure of the RIF-1 cells to 2% (vol/vol) dimethyl sulfoxide (DMSO) for 7 days induced the expression of additional functional, voltage-dependent K^+ channels; these gave rise to currents that were measurable after heating. In parallel, these cells became heat resistant. Addition of TEA to DMSO-treated cells blocked channels and returned the heat response almost to the pre-DMSO levels. Our data show a correlation between heat resistance and expression of K^+ channels. Because resistance to heat very likely relates to the heat shock response, our data suggest that activation of channels may be a very early event in initiation of the heat shock response.**

The heat sensitivity of mammalian cells is largely determined by the species from which the cells were originally derived (1). For example, human cells are far more resistant to heat than are rodent cells. It has occasionally been possible, however, to select heat-resistant cells from heat-sensitive populations. Stable, heat-resistant variants, however, have only rarely been obtained (2–6). We derived a series of heat-resistant strains from the murine RIF-1 line (6) by repeated cycles of heating and regrowth at 37°C. Several strains (e.g., TR-4, TR-5), except for their heat resistance, closely mimicked the parent line as shown by morphological and kinetic characteristics (6). Biochemical analysis showed that the heat-resistant cells differ from their parent cells in two major aspects: their plasma membranes contain more saturated fatty acids than do membranes from the parent line, and their constitutive expression of heat shock proteins (HSPs) is different (7).

Both RIF-1 cells and the heat-resistant variants can be made transiently more heat resistant by first giving them a conditioning heat treatment. This transient resistance is called thermotolerance (8, 9). The induction of thermotolerance is usually accompanied by synthesis of HSPs, and it has frequently been suggested that the HSPs are responsible for protecting the cells against heat damage. Indeed, studies using genetic manipulation to overproduce HSP27 or HSP70 have shown that such overproduction results in increased heat resistance (10, 11), although a limited amount of thermotolerance can also be induced in the absence of synthesis of measurable amounts of HSPs (12–16).

While the role and function of HSPs are under intense scrutiny, much less is known about the involvement of the plasma membrane in determining heat resistance (27) and its role in the heat shock response. A few studies do show such an association (17), and heat shock results in the breakdown of arachidonic acid and subsequent activation of several messenger cascades (18).

These results suggested to us that transmembrane signaling might play a role in the cells' response to heat shock. To test this hypothesis, we examined plasma membrane voltage-dependent currents in cells that had been exposed to hyperthermic temperatures. We examined such currents in heat-sensitive cells, in heat-resistant cells, and in sensitive cells made thermotolerant. In parallel, we tested the effect of a specific K^+ channel-blocking agent, tetraethylammonium (TEA), on the ability of cells to survive heat shocks. All our results are consistent with the view that cells able to develop sustained K^+ voltage-dependent currents in response to hyperthermic treatments are relatively resistant to exposures to elevated temperature.

MATERIALS AND METHODS

Cells. RIF-1 and TR-4 cells were grown in sterile 60-mm Nunc dishes (Intermed, Roskilde, Denmark) and incubated overnight in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS).

Whole Cell Current Recording. Whole cell currents were measured by the technique of Hamill *et al.* (19). The medium in which the cells were incubated was replaced with serum-free RPMI 1640 medium to ensure better seals. Glass electrodes with a tip resistance of 3–5 MΩ were treated with Sylgard and filled with a solution (pH 7.2) containing 140 mM KCl, 2 mM $MgCl_2$, 11 mM EGTA, 1 mM $CaCl_2$, and 10 mM Hepes. Membrane currents were filtered at 2–5 kHz using a 4-pole low-pass Bessel filter before sampling with a 12-bit digital/analog converter. These were controlled by a Labmaster (Scientific Solutions, Solon, OH) board installed in an IBM AT computer. Leakage currents were subtracted from the total current measured at each voltage either by using a P, P/4 algorithm (20) inbuilt into the software (Indec Systems, Santa Cruz, CA) or after determining the linear component from the current–voltage (*I–V*) relationship near the holding potential of −75 mV.

Incubation in Dimethyl Sulfoxide (DMSO). RIF-1 cells were grown in RPMI 1640 medium supplemented with 10% FBS and 2% (vol/vol) DMSO for 7 days. The medium was replaced with DMSO-free RPMI 1640 medium containing

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Abbreviations: HSP, heat shock protein; TEA, tetraethylammonium; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; IP3, inositol 1,4,5-trisphosphate.

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10% FBS and both voltage-dependent currents and surviving fractions in response to heat were measured.

Determination of Surviving Fraction. After overnight incubation of RIF-1 and TR-4 cells in RPMI 1640 medium containing 10% FBS, the medium was replaced with fresh RPMI 1640 medium containing 10% FBS, and the cells were heated at 45°C for various durations. The heated cells were treated with trypsin, counted, and plated into sterile 60-mm Nunc dishes. These were incubated at 37°C for 10 days. The colonies were stained and counted, and the surviving fractions were determined.

RESULTS AND DISCUSSION

We did not detect any currents in unheated RIF-1 and TR-4 cells; a minimum of 35 cells of each group was tested. However, we recorded currents immediately after heating at 45°C for 10 min (Fig. 1*A*) in TR cells (either TR-4 or TR-5). The *I–V* relationship (Fig. 1*C*) for the heated TR-4 cells indicated that we were measuring expressions of K^+ currents. We saw both delayed-rectifier and fast-inactivating currents. These subsequently decayed so that 6 h later they could no longer be detected. In contrast, heated RIF-1 cells (45°C for 10 min) displayed voltage-dependent currents only during the heating period. In the latter cells, the heat-induced voltage-dependent currents decayed to nondetectable levels immediately upon return to 37°C. In TR-4 cells, heating induced sustained voltage-dependent currents.

We then used a specific blocker of K^+ channels to test whether the currents were indeed potassium fluxes. TEA (20 mM), a blocker of K^+ channels, completely inhibited the currents (Fig. 1*D*). We further tested the nature of the currents by replacing the sodium cations in the bathing medium with K^+ and measuring the reverse potential. We observed a linear relationship between $\ln[K^+]$ and the reverse potential as predicted by the Nernst equation (Fig. 1*E*).

The transmembrane potential of TR-4 cells was -42 ± 5 mV ($n = 10$), while that of the RIF-1 cells was -41 ± 6 mV ($n = 20$). Upon heating for 10 min at 45°C, the transmembrane potentials of the TR-4 and RIF-1 cells were not significantly changed. The transmembrane potential of the heated TR-4 cells indicates that ≈200 pA of K^+ currents flowed through the activated voltage-dependent channels (Fig. 1*C*), while we detected no sustained voltage-dependent K^+ currents in heated RIF-1 cells. In neither case was there a significant change in the intracellular concentration of K^+ that might trigger a buffering action via the ATP-dependent Na^+/K^+ or K^+/H^+ pumps.

Because the heat dose used (10 min; 45°C) killed an appreciable fraction of the RIF-1 cells, but few, if any, of the TR cells, we repeated the experiment but heated the RIF-1 cells for only 3 min at 45°C, a dose that permits survival of at least 50% of these cells. Again, we saw no voltage-dependent

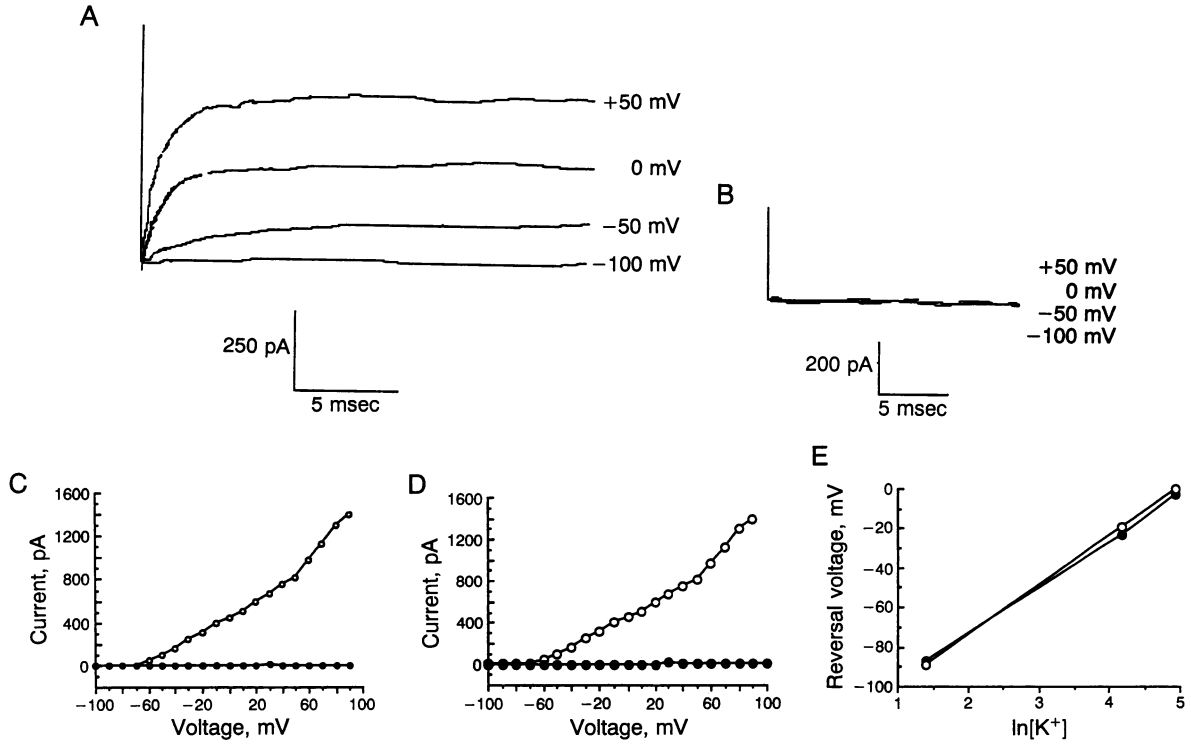

FIG. 1. Activation by heat shock of K^+ channels in TR-4 but not in RIF-1 cells. Cells (RIF-1 and TR-4 cells) were grown in sterile 60-mm Nunc dishes (Intermed) and incubated overnight in RPMI 1640 medium (GIBCO) supplemented with 10% FBS. (*A*) Current measured from a TR-4 cell 20 min after heating at 45°C for 10 min. Voltage steps to (from bottom to top) −100, −50, 0, and +50 mV were delivered every 100 msec from a 500-msec prepulse of −120 mV. The holding potential was −75 mV. (*B*) Current measured from a RIF-1 cell 20 min after heating at 45°C for 10 min. Note the change in scale from *A*. (*C*) The *I–V* relationship for currents recorded from TR-4 (○) and RIF-1 (●) cells 20 min after heating at 45°C for 10 min. Note the absence of currents in heated RIF-1 in contrast to heated TR-4 cells. The *I–V* relationship for the current recorded from the TR-4 cell suggests that K^+ voltage-dependent channels were being activated. (*D*) *I–V* relationship of current recorded from a preheated TR-4 cell (10 min at 45°C) before (○) and after (●) addition of 20 mM TEA. Heat-induced currents are sensitive to TEA, indicating that the measured currents were due to K^+ fluxes. (*E*) TR-4 cells were heated at 45°C for 30 min in RPMI 1640 medium supplemented with 10% FBS. The medium was replaced with the same high K^+ solution used to fill the glass electrode. A 100-msec voltage step to +50 mV from a holding voltage of −75 mV was followed by a 50-msec postpulse at +50 mV, during which the tail currents were recorded. The postpulse was decreased during subsequent cycles in steps of 10 mV to determine the reverse potential. The reverse potential was similarly determined for solutions containing various concentrations of K^+. The reverse potential in preheated TR-4 cells is linearly related to the $\ln[K^+]$ as predicted by the Nernst equation. ●, Measured; ○, theoretical.

currents in the heat-sensitive cells after heating. Conversely, we heated TR-4 cells at 45°C for 30 min (a dose resulting in a survival fraction equal to that observed when RIF-1 cells are heated at 45°C for 10 min) and measured higher voltage-dependent K^+ currents than were recorded for the 10-min heating of the TR-4 cells. The outward current for TR-4 cells at 0 mV after the 30-min heating was 952 ± 48 pA (n = 15) as compared to 430 ± 29 pA (n = 18) after 10-min heating at 45°C. In contrast, the outward current at 0 mV for RIF-1 cells during heating was 195 pA (n = 2).

Do the currents reflect survival-related phenomena? To examine this, we heated RIF-1 and TR cells in the presence of 20 mM TEA and measured cell survival as a function of duration of exposure at 45°C. The channel blocker had no effect on the heat sensitivity of the RIF-1 cells (Fig. 2). Cells from the TR strains were appreciably more heat sensitive in the presence of TEA (Fig. 2), suggesting a direct link between heat sensitivity and sustained K^+ current activation. TEA, however, did not quite reduce the survival of TR-4 cells to that of equivalently heated RIF-1 cells, suggesting that events initiated by activating K^+ channels do not fully account for the difference in heat resistance between these cells.

To test the relationship between heat resistance and K^+ currents further, we made RIF-1 cells resistant to heat in two ways. First, we preheated them at a nonlethal dose, and then waited 8 h for development of thermotolerance. Thermotolerant cells did not show voltage-dependent currents after a second heat challenge, nor did incubation of these cells in the presence of 20 mM TEA overcome thermotolerance (data not shown). The second method of inducing resistance involved growth of the cells in 2% DMSO (17). DMSO is also known to induce activation of K^+ channels in neuroblastoma N_2AB-1 cells (21). RIF-1 cells so grown for 7 days became both heat resistant (Fig. 3*A*) and developed sustained potassium currents (Fig. 3*B*). Addition of TEA to the medium abolished these K^+ currents (Fig. 3*B*) and reduced heat resistance (Fig. 3*C*). DMSO-treated cells transferred to DMSO-free medium for 7 days lost both their heat resistance and the sustained K^+ currents (data not shown). These results point to a clear difference between these two methods

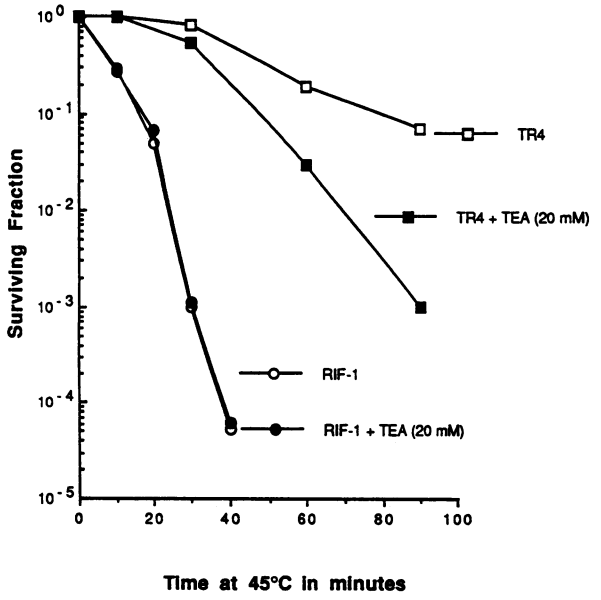

FIG. 2. Effect of TEA on cell survival after heat shock. RIF-1 cells and TR-4 cells were grown as described in Fig. 1*A*. Dishes containing the cells were heated at 45°C for various lengths of time with or without 20 mM TEA. Cells were treated with trypsin and plated using appropriate dilutions for colony formation. TEA sensitized TR-4 cells to heat but had no effect on RIF-1 cells, indicating that the heat-induced K^+ currents are heat resistant.

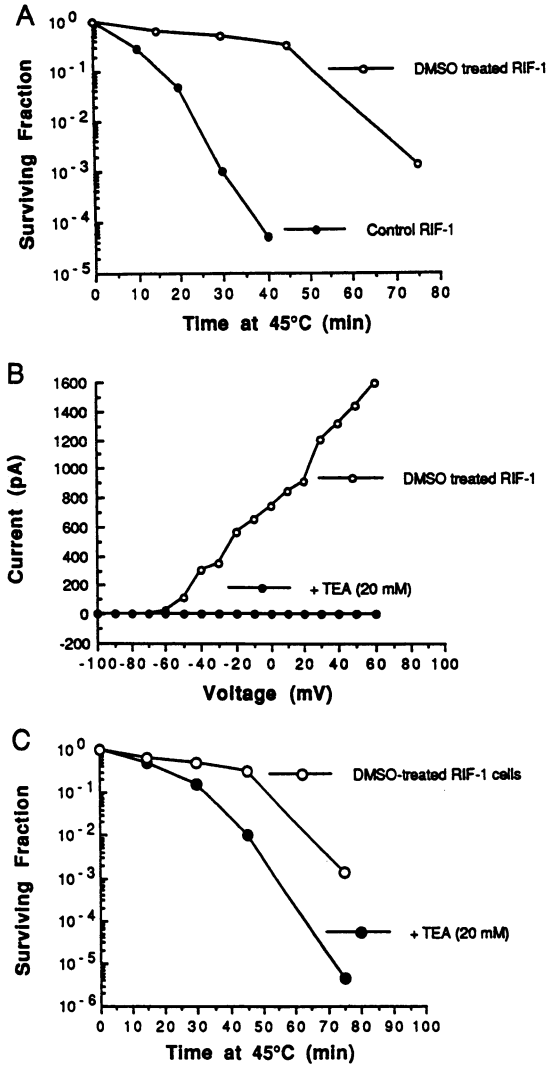

FIG. 3. Induction of heat resistance and activation of K^+ channels in RIF-1 cells treated with DMSO. RIF-1 cells were grown in RPMI 1640 medium supplemented with 10% FBS and 2% DMSO. After 7 days of growth, the medium was replaced by DMSO-free medium and both survival and voltage-dependent currents in response to heat were measured. (*A*) Survival fractions of cells after exposure to various lengths of time at 45°C. Note increased resistance over untreated cells. (*B*) *I–V* relationship for DMSO-treated cells. Note absence of currents in these cells in the presence of 20 mM TEA. (*C*) Presence of 20 mM TEA appreciably reduced heat resistance of the DMSO-treated cells.

of making cells heat resistant. Preheating is accompanied by induction of HSPs, while growth in DMSO results in a reduction of both the constitutive and induced levels of HSP70 (unpublished data). Preheating results in the development of transient heat resistance. This transient form of heat resistance is termed thermotolerance (8, 9). For RIF-1 cells, peak thermotolerance is achieved ≈8 h after a primary heat dose of 45°C for 10 min and returns to normal within 72 h. Heat-resistant TR-4 cells can also be made thermotolerant by heating at 45°C for 40 min, indicating a difference between thermotolerance and innate heat resistance. Thermotolerant cells do not reinitiate a heat shock response upon reheating. These results are therefore consistent with the view that initiation of K^+ currents relates to early events of the heat shock response.

How could K^+ channels be relevant to the expression of heat resistance and to the initiation of the heat shock response? Heat activates the inositol 1,4,5-trisphosphate (IP3)/Ca^{2+} signal transduction pathway, and Calderwood *et al.* (18)

showed that hyperthermia (45°C) caused the release of IP3 within 1 min of heating. Increases in levels of IP3 are followed shortly by an increase in the concentration of intracellular free Ca^{2+}. IP3-induced Ca^{2+} release can be triggered by the interaction of a guanine nucleotide binding protein (G protein) with a membrane ligand or receptor (22). K^+ channel blockers such as TEA inhibit the IP3-induced Ca^{2+} release (23). G proteins also directly couple membrane receptors and ligands to K^+ channels via a membrane-delimited, noncytoplasmic mechanism (24–26). Direct activation by heat of K^+ channel activity, as suggested by our data, may be another way of initiating messenger cascades and raises the intriguing possibility that channels act as the molecules able to sense changes in stress levels. Reinforcing this, in data to be shown elsewhere, we demonstrate that K^+ channel activity can be induced by oxidative stress and by low levels (10 cGy) of x-irradiation.

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